REMARKS

Status of the Claims

Pending claims

Claims 1 to 37 are pending (claims 1 to 30 as filed, 31 to 37 added in the response to the restriction requirement).

Response to the Restriction Requirement

In response to the Restriction Requirement mailed September 05, 2002, Applicants elected Group I, claims 1-17 and 22-24, drawn to methods of making alpha-substituted carboxylic acids using nitrilases, with traverse.

In response to the species election, Applicants elected: for the carboxylic acid produced, 2-chloro mandelic acid; for the nitrilase used, the polypeptide as set forth in SEQ ID NO:4 (which, in one aspect, is encoded by an sequence as set forth in SEQ ID NO:3); for the cyanide used, KCN (see page 77, line 23 of the specification); for the ammonium salt, NH4Cl (see page 77, line 24 of the specification).

Applicants requested the Patent Office to rejoin all claims directed to methods of making alpha -substituted carboxylic acids and the corresponding products of the methods, or alpha -substituted carboxylic acids, into one restriction group. Group I is drawn to methods of making alpha -substituted carboxylic acids using nitrilases. Groups III through VI are drawn to nitrilase polypeptides and nucleic acids which encode them (in one aspect, SEQ ID NOS:1 to 4).

Applicants thank the Examiner for noting that claims 31 to 37 are drawn to the elected invention. Accordingly, claims 1 to 17, 22 to 24 and 31 to 37 are pending and under consideration.

Applicants have preserved their right to petition the restriction

In their response of January 17, 2003, Applicants traversed the restriction requirement and respectfully requested the restriction be withdrawn. Applicants set forth distinct and specific errors in the restriction requirement and reasons for the Patent Office to reconsider and withdraw, in part, the restriction requirement. Accordingly, Applicants have preserved their right to petition the restriction to the Group Director under 37 CFR §1.144; see also MPEP

§818.03(c); pg 800-60, 8th Edition, August 2001. Applicants will defer submission of the petition (which can be deferred until allowance of the claims).

Claims amended and added in the instant amendment

In the present response and amendment, claims 1, 2, 10, 24 and 32 are amended; and new claims 38 to 44 are added. Thus, after entry of these amendments, claims 1 to 17, 22 to 24 and 31 to 44 are pending and presented for consideration.

Outstanding Rejections

Claims 1 to 17, 24 and 31 to 37 are rejected under 35 U.S.C. §112, second paragraph. Claims 1 to 17, 24 and 31 to 37 are rejected under 35 U.S.C. §112, first paragraph, written description. Claims 5 to 7 to 10 and 22 to 24 are rejected under 35 U.S.C. §112, first paragraph, enablement. Claims 1 to 4, 6, 11 to 17, 31 and 33 to 36 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto, U.S. Patent No. 5,587,303, as evidenced by Iyer (1996) Amino Acids 11:259-268. Claim 32 is rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto, U.S. Patent No. 5,587,303. Claims 32 and 37 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Bhalla (1992) Applied Micro. Biotech. 37:184-190. Applicants respectfully traverse all outstanding objections to the specification and rejections of the claims.

Information Disclosure Statement

Noting paragraphs 4 and 5, pages 3 to 4, of the office action, Applicants are submitting a supplementary Information Disclosure Statement and Form PTO-1449 under a separate cover.

Objections to the Specification

Title

In paragraph 6, page 4, of the instant Office Action, a new title is recommended. The instant amendment addresses this issue.

Abstract

In paragraph 7, page 4, of the instant Office Action, an amendment to the abstract is recommended. The instant amendment addresses this issue.

Specification

In paragraphs 8, 9, 10 and 11, pages 4 to 5, of the instant Office Action, the specification is objected to for various informalities. The instant amendment addresses these issues.

Objections to the Claims

In paragraph 12, page 5, of the instant Office Action, claim 10 is objected to. The instant amendment addresses this issue.

Support for the Claim Amendments

Support for the claim amendments can be found throughout the specification. For example, support for claims directed to methods using a nitrilase having an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75% or 70% sequence identity to an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4, and in one aspect, wherein the sequence identity is determined using a FASTA version 3.0t78 algorithm with default parameters can be found, e.g., on page 62, lines 7 to 24.

Issues under 35 U.S.C. §112, second paragraph

Claims 1 to 17, 24 and 31 to 37 are rejected under 35 U.S.C. §112, second paragraph.

The phrase "nitrilase or a polypeptide having nitrilase activity"

The phrase "nitrilase or a polypeptide having nitrilase activity" is alleged to be unclear in view of the different enzymes named nitrilases known in the art, and, in particular, that it is unclear if nitrilases from both class EC 3.5.5.1 and EC 4.2.1.84, or just one of these classes, are intended to be included within the scope of the claimed invention.

On page 6, lines 6 to 9, the specification describes which nitrilases are within the scope of the invention:

> Nitrilases contemplated for use in the practice of the present invention include those which are sufficiently robust to stereoselectively hydrolyze the transient amino

nitrile or cyanohydrin under Strecker conditions, i.e., in the presence of cyanide and ammonia. Such nitrilases include, for example, those set forth in SEQ ID Nos:2 and 4.

Accordingly, any nitrilase that can stereoselectively hydrolyze a transient amino nitrile or cyanohydrin under Strecker conditions is within the scope of the claimed invention, including nitrilases from both classes EC 3.5.5.1 and EC 4.2.1.84.

The structure "C"*

The structure "C*" is alleged to be unclear, i.e., the meaning of "*" in claim 2 is alleged to be unclear. The instant amendment addresses this issue (the asterisk has been deleted).

The phrase "optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety"

The phrase "optionally R_1 and R_2 are linked to cooperate to form a functional cyclic moiety" is alleged to be unclear. The instant amendment addresses this issue.

The phrases "amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4" and "a nucleic acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3"

The phrases "amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4" and "a nucleic acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3" are alleged to be unclear. The phrases "amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4" and "a nucleic acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3" as used in claims 22 to 24 do not include "sequences substantially identical thereto."

The phrase "intermediate of step (a)"

The phrase "intermediate of step (a)" is alleged to be unclear. The instant amendment addresses this issue.

Issues under 35 U.S.C. §112, first paragraph

Written Description

Claims 1 to 17, 24 and 31 to 37 are rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time

the application was filed had possession of the invention. This is a section 112, first paragraph written description rejection.

In particular, the Patent Office alleges that the instant claims are drawn to methods of making alpha-carboxylic acids using nitrilases, wherein either no structure or limited structure, as in claim 24 (see also new claim 44), is noted to distinguish the nitrilases application to the instant claims. The Patent Office cites University of California v. Eli Lilly & Co., 43 USPQ2d 1398 (Fed. Cir. 1997).

Claim 1, after entry of the instant amendment, reads:

> A method for producing an enantiomerically pure α-substituted carboxylic acid, said method comprising contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or polypeptide having a nitrilase activity, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid.

Applicants respectfully note that this method is not limited to using the novel nitrilases of the invention. Similarly, the methods of claims 31, 32 and 36 are not limited to using the novel nitrilases of the invention. Claims 1, 31, 32 and 36 are sufficiently broad to encompass all known nitrilases, including the novel nitrilases of the invention, capable of stereoselectively hydrolyzing amino nitrile or cyanohydrin intermediates to produce an enantiomerically pure α-substituted carboxylic acid.

In contrast, University of California v. Eli Lilly & Co., 43 USPQ2d 1398 (Fed. Cir. 1997), addresses written description issues for claiming a novel genetic material (e.g., novel nucleic acid or polypeptide sequences). Nitrilases that can be used to practice the methods of claims 1, 31, 32 and 36, include those known in the art. Thus, written description issues for claiming a novel genetic material are not applicable to the methods of claims 1, 31, 32 and 36, and the nitrilases used to practice these methods.

However, with regard to claims 22 to 24 and 38 to 44, directed to methods that incorporate use of the novel nitrilases of the invention, Applicants respectfully aver that the claims fully comply with the requirements for written description of a genus of nucleic acids. In University of California v. Eli Lilly & Co., 43 USPQ2d 1398 (Fed. Cir. 1997), the Federal

Circuit stated that, "[a] description of a genus of cDNA may be achieved by means of a recitation of a representative number of cDNAs....*or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus.*" (emphasis added) Lilly, 43USPQ2d at 1406.

The invention's novel nucleic acids and polypeptides (e.g., the exemplary SEQ ID NO:1, SEQ ID NO:3, and, SEQ ID NO:2 and SEQ ID NO:4, respectively) used in the methods of the invention are described by structure (the exemplary nucleic acid or polypeptide sequence), a physico-chemical property (e.g., percent sequence identity) and function (nitrilase activity). The specification discloses exemplary sets of parameters that provided the skilled artisan with the physical/chemical properties of the invention's novel nucleic acids and polypeptides as well as providing a function for them. Accordingly, the specification adequately describes the invention of claims 22 to 24 and 38 to 44, to satisfy the written description requirements of section 112, first paragraph.

The instant claims clearly set forth specific structural and physical characteristics of the novel nitrilases used in the methods of the invention. The genus of polypeptides used in the claimed methods all must have a nitrilase activity and a specific physical characteristic, e.g., a specific sequence identity, to the exemplary polypeptide sequence. Therefore, the sequences used in the methods of claims 22 to 24 and 38 to 44, are defined via shared physical and structural properties in terms that "convey with reasonable clarity to those skilled in the art that Applicant, as of filing date sought, was in possession of invention." Vas-Cath Inc. V. Mahukar, 19 USPQ2d 1111 (Fed Cir. 1991).

More recently, the Federal Circuit stated

> Similarly, in this court's most recent pronouncement, it noted:
>
> > More recently, in Enzo Biochem, we clarified that Eli Lilly did not hold that all functional descriptions of genetic material necessarily fail as a matter of law to meet the written description requirement; rather, the requirement may be satisfied if in the knowledge of the art the disclosed function is sufficiently correlated to a particular, known structure.
>
> Amgen, 314 F.3d at 1332 [Amgen Inc. v. Hoechst Marion Roussel Inc., 314 F.3d 1313, 1330, 65 USPQ2d 1385, 1397 (Fed. Cir. 2003)].

Moba, B.V. v. Diamond Automation, Inc., 2003 U.S. App. LEXIS 6285; Fed. Cir. 01-1063, -1083, April 1, 2003.

Analogously, the disclosed function of the nitrilases of the instant invention used in the claimed methods is sufficiently correlated to a particular, known structure (the exemplary sequences) and a physical (physico-chemical) property (sequence identity). Accordingly, the claimed sequences are defined via shared physical and structural properties in terms that convey with reasonable clarity to those skilled in the art that Applicants, as of the filing date and at the time of the invention, were in possession of the claimed invention.

Further with respect to satisfying the written description requirement for sequences based on sequence identity, Applicants respectfully refer to the USPTO guidelines concerning compliance with the written description requirement of U.S.C. §112, first paragraph. In example 14 of the guidelines (a copy of which is attached as Exhibit A), a claim reciting variants claimed by sequence identity to a sequence is sought (specifically, "A protein having SEQ ID NO:3 and variants thereof that are at least 95% identical to SEQ ID NO:3 and catalyze the reaction of A → B). In the example, the specification is described as providing SEQ ID NO:3 and a function for the protein. The specification contemplates, but does not exemplify variants of SEQ ID NO:3 that can have substitutions, deletions, insertions and additions. Procedures for making proteins with substitutions, deletions, insertions, and additions are routine in the art and an assay is described which will identify other proteins having the claimed catalytic activity. The analysis of example 14 states that procedures for making variants (which have 95% sequence identity) are conventional in the art. The Guidelines conclusion states that the disclosure meets the requirements of 35 U.S.C. §112, first paragraph, as providing adequate written description for the claimed invention.

Analogously, the claimed nucleic acids are described by structure (the exemplary sequence), a physico-chemical property (percent sequence identify) and function (nitrilase activity). The USPTO guidelines recognize that written description is met for a genus of polypeptides described by structure, a physico-chemical property (e.g., a % sequence identity, stringent hybridization) and a defined function, the genus of claimed polypeptides also meet the written description requirements of section 112.

Applicants also respectfully note that claims directed to a genus of polypeptide-encoding nucleic acids or polypeptides as described and enabled by the specific physical characteristic of percent sequence identity or stringent hybridization and function have been issuing from the USPTO recently and for many years, see, e.g., U.S. Patent Nos. 6,541,684; 6,541,236; 6,541,220; 6,534,309; 6,492,150; 6,465,210; 6,413,522; 6,384,304; 6,342,657; 6,274,790 (selected claims from these patents are attached as Exhibit B).

Accordingly, Applicants respectfully submit that the claimed methods using the novel nitrilases of the invention meet the written description requirement under 35 U.S.C. §112, first paragraph.

The Patent Office also alleges that one skilled in the art would be unable to identify the structures of other nitrilases [other than the exemplary nitrilases] useful in the claimed methods by virtue of the instant disclosure (see page 8, the first full paragraph, of the office action).

Applicants respectfully aver that the nitrilases used to practice the methods of the invention are either known in the art or include the novel nitrilases of the invention whose structures, as discussed above, are sufficiently described to satisfy the written description requirements of section 112, first paragraph.

The issue of whether one skilled in the art would be able to identify other nitrilases (other than the exemplary nitrilases) useful in the claimed methods by virtue of the instant disclosure is a question of enablement encompassing undue experimentation issues is discussed below.

The Patent Office alleges that because there is not sufficient description of various chemical compounds (as specifically listed on page 9, first full paragraph) the claims lack sufficient written description in the specification. However, Applicants respectfully aver that because these chemical compounds (e.g., D-phenylalanine, (S)-cyclohexylmadelic acid, alpha-amino nitrile, etc.) were well known in the art at the time of the invention, it was not necessary to list their specific structures in the specification.

Enablement

Claims 5 to 7 to 10 and 22 to 24 are rejected under 35 U.S.C. §112, first paragraph, as allegedly not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. In particular, it is alleged that the identification of nitrilases that perform the proper hydrolysis reaction on the proper substrate would require undue experimentation.

Applicants respectfully maintain that the specification enabled the skilled artisan at the time of the invention to identify, and make and use, a genus of nitrilases to practice the claimed methods. The state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art, e.g., screening enzymes, and nucleic acids encoding enzymes, for nitrilase activity such that the enzyme produces an enantiomerically pure alpha-substituted carboxylic acid, was very high. Accordingly, it would not have taken undue experimentation to make and use the claimed invention, including identification of nitrilases that perform the proper hydrolysis reaction on the proper substrate.

In fact, whether large numbers of compositions (e.g., enzymes, antibodies, nucleic acids, and the like) must be screened to determine if one is within the scope of the claimed invention is irrelevant to an enablement inquiry. Enablement is not precluded by the necessity to screen large numbers of compositions, as long as that screening is "routine," i.e., not "undue," to use the words of the Federal Circuit. As the Patent Office correctly notes, the Federal Circuit in In re Wands directed that the focus of the enablement inquiry should be whether the experimentation needed to practice the invention is or is not "undue" experimentation. The court set forth specific factors to be considered.

One of these factors is "the quantity of experimentation necessary." Guidance as to how much experimentation may be needed and still not be "undue" was set forth by the Federal Circuit in, e.g. Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987). In Hybritech, Inc., a single deposited antibody producing cell line enabled a claim generic to all IgM antibodies directed to a specific antigen. The Federal Circuit noted that the evidence indicated that those skilled in the monoclonal antibody art could, using the state of the art and applicants' written disclosure, produce and screen new hybridomas secreting other monoclonal antibodies falling within the

genus without undue experimentation. The court held that applicants' claims need not be limited to the specific, single antibody secreted by the deposited hybridoma cell line (significantly, the genus of antibodies was allowed even though only one antibody specie was disclosed). The court was acknowledging that, because practitioners in that art are prepared to screen large numbers of negatives in order to find a sample that has the desired properties, the screening that would be necessary to make additional antibody species was not "undue experimentation."

Analogously, practitioners of the biological sciences for the instant invention also recognize the need to screen numbers of negatives to find a sample that has the desired properties, e.g., nitrilases that perform the proper hydrolysis reaction on the proper substrate. Furthermore, the screening procedures used to identify nucleic acids within the scope of the instant invention were all well known in the art and at the time this application was filed. All were routine protocols for the skilled artisan. Thus, the skilled artisan using Applicants' written disclosure could practice the instant claimed invention without undue experimentation.

Additionally, the instant specification provides an exemplary method for screening for nitrilase activity, see, e.g., Example 1, pages 77 to 78. The routine protocol of Example 1 describes how to identify a nitrilase which can stereoselectively hydrolyze an amino nitrile or cyanohydrin intermediate to produce an enantiomerically pure alpha-substituted carboxylic acid. This is protocol was used to identify the exemplary nitrilases of the invention having sequences as set forth in SEQ ID NO:2 and SEQ ID NO:4. Accordingly, Applicants respectfully submit that the specification does reasonably provide one of ordinary skill in the art to make and use the subject matter of the claimed invention.

With the guidance provided in the specification, *e.g.*, a routine protocol to identify nitrilases that can produce an enantiomerically pure alpha-substituted carboxylic acid, it would only have required the skilled artisan routine experimentation to practice the full scope of the claimed invention. Accordingly, Applicants respectfully submit that the specification enables one of ordinary skill in the art to practice the full scope of the methods of the invention.

Declaration under Rule 132

Applicants submit a declaration under Rule 132 by co-inventor Dr. Jennifer Ann Chaplin attesting that an enantiomerically pure (S)-phenylglycine (yield of 58.8% and ee for (S)-

phenylglycine of 91.3% as measured by HPLC) was produced using a protocol within the scope of the invention, i.e., a method for producing an enantiomerically pure α-substituted carboxylic acid by contacting an aldehyde or ketone with a cyanide-containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing a resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or polypeptide having a nitrilase activity, where the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid. The details of the protocol are set forth in the declaration.

Dr. Chaplin declares that that using the teaching of the specification, including the exemplary protocol as set forth in Example 1, pages 77 and 78, of specification, and other protocols known in the art at the time of the invention, alternative protocols, including the protocol described below, could have been designed by one skilled in the art at the time of the invention to successfully practice the methods of the invention, i.e., to produce an enantiomerically pure a-substituted carboxylic acid by contacting an aldehyde or ketone with a cyanide-containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing a resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or polypeptide having a nitrilase activity, where the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure a-substituted carboxylic acid.

Dr. Chaplin also declares that at the time of the invention, with the teaching of the specification, it would have taken only routine screening by one skilled in the art to identify recombinant nitrilase enzymes capable of producing an enantiomerically pure alpha-substituted carboxylic acid by combining an aldehyde or ketone with a cyanide and ammonia or an ammonium salt or an amine.

Dr. Chaplin also notes that in reviewing the specification for preparing for this declaration, it was noticed that an inadvertent error was made in the protocol as set forth in

Example 1 on page 76 of the specification. The cell lysates contained nitrilase which had been overexpressed in an *E. coli* host, not a *Pseudomonas* host (see line 4 of page 76).

Additional comments

On page 8, lines 13 to 16, of the Office Action the Patent Office states "It can be inferred from Example 1 and the sequence listing that the proteins were isolated from an environmental sample, sequenced, used to probe for DNA sequences that were then made recombinantly and expressed in *Pseudomonas* host ... ."

For the record, Applicants respectfully note that they discovered the nitrilases used in the claimed methods (SEQ ID NO:2, encoded by SEQ ID NO:1, and SEQ ID NO:4, encoded by SEQ ID NO:3) from DNA isolated from environmental samples. The nucleic acid was subcloned into *Pseudomonas* and overexpressed. Cell lysates were produced to run the experiments set forth in Example 1. The results from the experiment of Example 1 confirmed nitrilase activity of these enzymes and showed that the method can be used to show nitrilase activity of other enzymes.

Issues under 35 U.S.C. §102

Claims 1 to 4, 6, 11 to 17, 31 and 33 to 36 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto, U.S. Patent No. 5,587,303, issued December 24, 1996 (hereinafter "Wakamoto"), as evidenced by Iyer (1996) Amino Acids 11:259-268 (hereinafter "Iyer"). Claim 32 is rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto, U.S. Patent No. 5,587,303. Claims 32 and 37 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Bhalla (1992) Applied Micro. Biotech. 37:184-190 (hereinafter "Bhalla").

The legal standard for anticipation under 35 U.S.C. §102 is one of strict identity. To anticipate a claim, a single prior source must contain each and every limitation of the claimed invention. In re Paulson, 30 F.3d 1475, 1478-79, 31 USPQ2d 1671, 1673 (Fed. Cir. 1994)(citing In re Spada, 911 F.2d 705, 708, 15 USPQ2d 1655, 1657 (Fed. Cir. 1990)).

Wakamoto, as evidenced by Iyer

Claims 1 to 4, 6, 11 to 17, 31 and 33 to 36 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto, as evidenced by Iyer.

The Patent Office notes that Wakamoto teaches production of α-aminonitriles using the Strecker process and teaches production of α-aminonitriles with microorganisms or enzyme extracts of those microorganisms having nitrile-hydrolyzing activity. Column 13, lines 12 to 17, of Wakamoto reads

> In these reaction processes, it is also feasible to use destructed products of proliferated cells, dried cells, enzyme preparations such as cell free extract and purified nitrile-hydrolyzing enzymes, or cells and enzyme preparations immobilized according to conventional procedures.

Claim 1, after entry of the instant amendment, reads:

> A method for producing an enantiomerically pure α-substituted carboxylic acid, said method comprising contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a recombinantly generated nitrilase or polypeptide having a nitrilase activity, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the enantiomerically pure α-substituted carboxylic acid.

Wakamoto does not teach a method using recombinantly generated nitrilases or recombinantly generated polypeptides having nitrilase activity to produce an enantiomerically pure α-substituted carboxylic acid. Accordingly, because Wakamoto is not a single prior source that contains each and every limitation of the claimed invention, it cannot anticipate the claimed invention.

The Patent Office also notes that Wakamoto does not teach the specifics of the well-known Strecker process. Because Wakamoto does not teach this limitation, it cannot be a single prior source that contains each and every limitation of the claimed invention, and thus cannot anticipate the claimed invention.

Iyer is cited for allegedly teaching the Strecker process as reacting cyanide with an aldehyde or ketone and ammonia to produce aminonitriles. However, Iyer does not teach a method using a nitrilases or polypeptides having nitrilase activity to produce enantiomerically pure α-substituted carboxylic acids. Accordingly, because Iyer is not a single prior source that contains each and every limitation of the claimed invention, it cannot anticipate the claimed invention.

Wakamoto

Claim 32 is rejected under 35 U.S.C. §102(b) as allegedly anticipated by Wakamoto. It is alleged that Wakamoto teaches contacting α-aminonitriles with microorganisms or enzyme extracts of those microorganisms having nitrile-hydrolyzing activity, and that this contact produces the corresponding L-amino acids with enantiomeric purity.

Claim 32, after entry of the instant amendment, reads:

> A method for producing an alpha-substituted carboxylic acid, the method comprising
> (a) providing a composition comprising an amino nitrile or a cyanohydrin;
> (b) providing a composition comprising a recombinantly generated nitrilase or a polypeptide having a nitrilase activity; and
> (c) contacting the amino nitrile or cyanohydrin of step (a) with the composition of step (b) such that the nitrilase or polypeptide having nitrilase activity hydrolyzes the amino nitrile or cyanohydrin intermediate to produce an alpha-substituted carboxylic acid.

However, as noted above, Wakamoto does not teach a method using recombinantly generated nitrilases or recombinantly generated polypeptides having nitrilase activity to produce an enantiomerically pure α-substituted carboxylic acid. Accordingly, because Wakamoto is not a single prior source that contains each and every limitation of the claimed invention, it cannot anticipate the claimed invention.

Bhalla

Claims 32 and 37 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Bhalla. It is alleged that Bhalla teaches the reaction of various α-amino acids with a nitrilase from *Rhodococcus* to produce their corresponding amino acids (noting abstract).

However, Bhalla does not teach a method using recombinantly generated nitrilases or recombinantly generated polypeptides having nitrilase activity to produce an enantiomerically pure α-substituted carboxylic acid. Accordingly, because Bhalla is not a single prior source that contains each and every limitation of the claimed invention, it cannot anticipate the claimed invention.

CONCLUSION

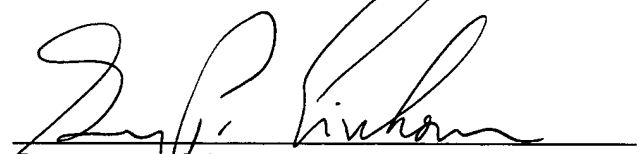
In view of the foregoing amendment and remarks, it is believed that the Examiner can properly withdraw the rejection of the pending claims under 35 U.S.C. §112, first and second paragraphs and 35 U.S.C. §102. Applicants believe all claims pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Applicants believe that no additional fees are necessitated by the present response and amendment. However, in the event any such fees are due, the Commissioner is hereby authorized to charge any such fees to Deposit Account No. 06-1050. Please credit any overpayment to this account.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (858) 678-5070.

Respectfully submitted,

Date: July 17, 2003



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Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A → B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

Exhibit B

United States Patent **6,410,264**
Crawford , et al. **June 25, 2002**

Pichia pastoris gene sequences and methods for their use

Abstract

Regulatory nucleotide sequences for a novel Pichia pastoris gene, designated PpSEC10 gene, and the nucleotide sequences and respective amino acid sequences for the secretion leader and the mature Sec10p protein components of the precursor polypeptide encoded by this novel gene are provided. These compositions are useful in methods for expression and secretion of proteins when assembled in proper reading frame, individually or in combination, within a DNA construct that further comprises a nucleotide sequence encoding a protein of interest. Vectors comprising the DNA constructs of the invention can be used to transform a yeast host cell, which can then be cultured to obtain the secreted protein of interest. Kits useful in this method and in methods of detection of the Sec10p protein using antibodies are also disclosed.

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Intern'l Class: C12Q 001/68
Field of Search: 935/72,33 435/69.1,6,5,91.1,172.3,320.1

References Cited [Referenced By]

U.S. Patent Documents

5215898	Jun., 1993	Bolen et al.
5324639	Jun., 1994	Brierley et al.
5324660	Jun., 1994	Gleeson et al.
5521086	May., 1996	Scott et al.
5602034	Feb., 1997	Tekamp-Olson.

Foreign Patent Documents

0 495 208	Jul., 1992	EP.
WO94/21802	Sep., 1994	WO.
WO96/40776	Dec., 1996	WO.
WO97/12044	Apr., 1997	WO.

Other References

Genbank Database, Accession No. U52430, May 28, 1996, W.E. Payne and C.A. Keiser,

"PpSEC13p".
Romanos, Michael A., et al., "Foreign Gene Expression in Yeast: A Review", Yeast, Jun. 1992, pp. 423-488, vol. 8, John Wiley & Sons Ltd.
Cregg, James M., et al., "Recent Advances in the Expression of Foreign Genes in Pichia pastoris", Bio/Technology, Aug. 1993, pp. 905-910, vol. 11.
Payne, William E., et al., "An Inducible Acid Phosphatase from the Yeast Pichia pastoris: Characterization of the Gene and Its Product", Gene, 1995, pp. 19-26, vol. 163, Elsevier Science B.V.
Sreekrishna, Koti, et al., "Strategies for Optimal Synthesis and Secretion of Heterologous Proteins in the Methylotrophic Yeast Pichia pastoris", Gene, 1997, pp. 55-62, vol. 190, Elsevier Science B.V.
Buckholz, Richard G., "Yeast Systems for the Expression of Heterologous Gene Products", Current Opinion in Biotechnology, 1993, pp. 538-542, vol. 4, Current Biology Ltd.
Busser, K., et al., "Heterologous Protein Expression in Pichia pastoris", American Biotechnology Laboratory, 1993, pp. 10-EOA, vol. 11, No.13, International Scientific Communications.

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Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 60/054783, filed Aug. 5, 1997, and U.S. Provisional Application Ser. No. 60/069560, filed Dec. 12, 1997, the contents of which are herein incorporated by reference.

Claims

That which is claimed:

1. A recombinant construct comprising in proper reading frame a nucleotide sequence for a promoter and a nucleotide sequence encoding a polypeptide, where said promoter drives transcription of an operably linked nucleotide sequence of interest, wherein said nucleotide sequence for said promoter is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 2;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 2;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 2; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

2. The construct of claim 1, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2.

3. The construct of claim 1, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acid residues of an amino acid sequence for said human IGF-I.

4. The construct of claim 1, further comprising a nucleotide sequence encoding a functional: secretion leader, wherein said nucleotide sequence encoding said secretion leader is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4;

b) the nucleotide sequence set forth in SEQ ID NO: 5;

c) a nucleotide sequence encoding an amino acid sequence having at least 70% ***sequence identity*** to the amino acid sequence set forth in SEQ ID NO: 4;

d) a nucleotide sequence encoding at least 8 contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO: 4; and

e) a nucleotide sequence that hybridizes to any of a), b), c), or d) under conditions of high stringency.

5. The construct of claim 4, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID) NO: 4.

6. The construct of claim 1, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

7. The construct of claim 6, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence for said terminator is the sequence set forth in SEQ ID NO: 3.

8. The construct of claim 4, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

9. The construct of claim 8, wherein said nucleotide sequence for said promoter is the sequence set forth in SEQ ID NO: 2, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4, and wherein said nucleotide sequence for said transcription terminator is the sequence set forth in SEQ ID NO: 3.

10. The construct of claim 8, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acid residues of an amino acid sequence for said human IGF-I.

11. A recombinant construct comprising in proper reading frame a nucleotide sequence for a yeast-recognized promoter, a nucleotide sequence encoding a functional secretion leader, and a nucleotide sequence encoding a polypeptide, wherein said nucleotide sequence encoding said secretion leader is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4;

b) the nucleotide sequence set forth in SEQ ID NO: 5;

c) a nucleotide sequence encoding an amino acid sequence having at least 70% ***sequence identity*** to the amino acid sequence set forth in SEQ ID NO: 4;

d) a nucleotide sequence encoding at least 8 contiguous amino acid residues of the amino acid sequence set forth in SEQ ID NO: 4; and

e) a nucleotide sequence that hybridizes to any of a), b), c), or d) under conditions of high stringency.

12. The construct of claim 11, wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4.

13. The construct of claim 11, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 continuous amino acids residues of an amino acid sequence for said human IGF-I, and wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4.

14. The construct of claim 11, further comprising a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

15. The construct of claim 14, wherein said nucleotide sequence encoding said secretion leader is a nucleotide sequence encoding the amino acid sequence set forth in SEQ ID NO: 4, and wherein said nucleotide sequence for said transcription terminator is the sequence set forth in SEQ ID NO: 3.

16. A recombinant construct comprising in proper reading frame a nucleotide sequence for a yeast-recognized promoter, a nucleotide sequence encoding a polypeptide, and a nucleotide sequence for a functional transcription terminator, wherein said nucleotide sequence for said terminator is selected from the group consisting of:

a) the nucleotide sequence set forth in SEQ ID NO: 3;

b) a nucleotide sequence having at least 70% ***sequence identity*** to the nucleotide sequence set forth in SEQ ID NO: 3;

c) a nucleotide sequence comprising at least 24 contiguous nucleotides of the sequence set forth in SEQ ID NO: 3; and

d) a nucleotide sequence that hybridizes to any one of a), b), or c) under conditions of high stringency.

17. The construct of claim 16, wherein said nucleotide sequence for said terminator is the sequence set forth in SEQ ID NO: 3.

18. The construct of claim 16, wherein said polypeptide is selected from the group consisting of human IGF-I, a polypeptide having at least 70% ***sequence identity*** to said human IGF-I, and a fragment of said human IGF-I, wherein said fragment comprises at least 10 contiguous amino acids of an amino acid sequence for said human IGF-I.

19. A vector comprising at least one copy of the construct of claim 1.

20. The vector of claim 19, wherein said vector is an autonomously replicating vector.

21. The vector of claim 19, wherein said vector is an integrative vector.

22. A vector comprising at least one copy of the construct of claim 8.

23. A vector comprising at least one copy of the construct of claim 11.

24. A vector comprising at least one copy of the construct of claim 16.

25. A yeast host cell stably transformed with at least one copy of the construct of claim 1.

26. The yeast host cell of claim 25, wherein said yeast is selected from the group consisting of Pichia

United States Patent **6,384,304**
Quandt , et al. **May 7, 2002**

Conditional sterility in wheat

Abstract

The invention relates to the use of a deacetylase coding sequence for obtaining conditional sterility in wheat. The invention relates to vectors comprising a deacetylase coding sequence under control of promoters which direct stamen-selective expression in wheat, which are particularly suited for the production of wheat plants which can be made male-sterile upon application of an acetylated toxin.

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Intern'l Class:	C12N 015/82; C12N 015/31; C12N 005/04; A01H 005/00; A01H 001/02
Field of Search:	800/320.3,288,300,287,274,303,271,278 435/418,419,468

References Cited [Referenced By]

U.S. Patent Documents

5633441	May., 1997	DeGreef et al.	800/205.
5650310	Jul., 1997	Broer et al.	435/172.
5668297	Sep., 1997	Broer et al.	800/205.
5689041	Nov., 1997	Mariani et al.	800/205.
5723763	Mar., 1998	Mariani et al.	800/205.
5767370	Jun., 1998	Broer et al.	800/205.
5767371	Jun., 1998	Broer et al.	800/205.
5767374	Jun., 1998	DeGreef et al.	800/205.
5792929	Aug., 1998	Mariani et al.	800/205.
5981189	Nov., 1999	Chan et al.	435/6.
6177616	Jan., 2001	Bartsch et al.	800/303.

Foreign Patent Documents

344029	Nov., 1989	EP.
WO 98/13504	Apr., 1998	WO.
WO 98/27201	Jun., 1998	WO.
WO 98/39462	Sep., 1998	WO.

Other References

Kriete G. et al., "Male sterility in transgenic tobacco plants induced by tapetum-specific deacetylation of the externally appliec non-toxic comound N-acetyl-L-phosphinothricin." 1996, The Plant Journal, vol. 9, pp. 809-818.*
"The development of a nuclear male sterility system in wheat. Expression of the barnase gene under the control of tapetum specific promoters"; DeBlock et al., 1997, Theor. Genet. 95: 125-131.

Primary Examiner: Fox; David T.
Attorney, Agent or Firm: Frommer Lawrence & Haug LLP

Claims

We claim:

1. A wheat plant, having a chimeric gene integrated into its genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp; and

b) a promoter directing stamen-selective expression in wheat wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under control of the stamen-selective promoter.

2. The wheat plant of claim 1, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ ID No. 9.

3. The wheat plant of claim 1, wherein said DNA molecule encodes a deacetylase having the amino acid sequence of SEQ ID No. 8.

4. The wheat plant of claim 1, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

5. The wheat plant of claim 1, wherein said stamen-selective promoter is a CA55 promoter.

6. The wheat plant of claim 1, wherein said stamen-selective promoter is a T72 promoter.

7. The wheat plant of claim 1, wherein said stamen-selective promoter is an E1 promoter.

8. A process for producing ***hybrid*** wheat seed, said process comprising

i) producing seeds capable of growing into conditionally male-sterile wheat plants, said seeds having a chimeric gene integrated in their genome, the chimeric gene comprising:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of the stamen-selective promoter;

ii) interplanting said seeds capable of growing into conditionally male-sterile wheat plants with seeds capable of growing into male fertile wheat plants;

iii) inducing male-sterility in said conditionally male-sterile plants by applying an N-acetyl-PPT, which in itself is not toxic to the plants or plant cells; and

iv) harvesting ***hybrid*** seed.

9. The process of claim 8, wherein said DNA molecule encodes a biologically active fragment or a variant of the deacetylase encoded by SEQ. ID. No. 9.

10. The process of claim 8, wherein said DNA molecule encodes the deacetylase comprising the amino acid sequence of SEQ ID No. 8.

11. The process of claim 8, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

12. The process of claim 8, wherein said stamen-selective promoter is a CA55 promoter.

13. The process of claim 8, wherein said stamen-selective promoter is a T72 promoter.

14. The process of claim 8, wherein said stamen-selective promoter is an E1 promoter.

15. The process of claim 8, wherein said male fertile plants are female-sterile.

16. A process for producing a conditionally male-sterile wheat plant, said process comprising

i) transforming a wheat plant cell or tissue with a chimeric gene which comprises:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of said stamen-selective promoter;

ii) regenerating said conditionally male-sterile plant from said cell or tissue; and optionally,

iii) applying an N-acetyl-PPT to said conditionally male-sterile plant, which is in itself not toxic to the plant or plant cells to make said plant male-sterile.

17. The process of claim 16, wherein said DNA molecule encodes a biologically active fragment of the deacetylase encoded by SEQ. ID. No. 9.

18. The process of claim 16, wherein said DNA molecule encodes the deacetylase of SEQ ID No. 8.

19. The process of claim 16, wherein said DNA molecule comprises the sequence of SEQ ID No. 9, or a sequence capable of hybridizing to SEQ ID No. 9 under standard ***stringent*** conditions.

20. The process of claim 16, wherein said stamen-selective promoter is a CA55 promoter.

21. The process of claim 16, wherein said stamen-selective promoter is a T72 promoter.

22. The process of claim 16, wherein said stamen-selective promoter is an E1 promoter.

23. A conditionally male sterile wheat plant obtained by the process of claim 16.

24. A process for generating male sterility in wheat plants, said process comprising

i) obtaining a conditionally male-sterile wheat plant by transforming a wheat plant cell or tissue with a chimeric gene which comprises:

a) a DNA molecule encoding a deacetylase from Stenotrophomonas sp., and

b) a stamen-selective promoter wherein the DNA molecule encoding the deacetylase is in the same transcriptional unit and under the control of said stamen-selective promoter, and regenerating said conditionally male-sterile plant from said cell or tissue; and optionally obtaining conditionally male-sterile progeny from said plant

ii) applying an N-acetyl-PPT to said conditionally male-sterile plant or its conditionally male-sterile progeny, which is in itself not toxic to the plant or plant cells to make said plant male-sterile.

25. A male sterile wheat plant, or cells or tissues thereof, obtained by the process of 24.

26. Wheat plant cells, tissues or seed, each transformed with the chimeric DNA of claim 1.

United States Patent **6,417,428**
Thomashow , et al. **July 9, 2002**

Plant having altered environmental stress tolerance

Abstract

A transformed plant is provided which comprises one or more environmental stress tolerance genes; a DNA regulatory sequence which regulates expression of the one or more environmental stress tolerance genes; a sequence encoding a binding protein capable of binding to the DNA regulatory sequence and inducing expression of the one or more environmental stress tolerance genes; and a recombinant promoter which regulates expression of the gene encoding the binding protein. A method for altering an environmental stress tolerance of a plant is also provided which comprises the steps of transforming a plant with a promoter which regulates expression of at least one copy of a gene encoding a binding protein capable of binding to a DNA regulatory sequence which regulates one or more environmental stress tolerance genes in the plant; expressing the binding protein encoded by the gene; and stimulating expression of at least one environmental stress tolerance gene through binding of the binding protein to the DNA regulatory sequence.

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Field of Search: 435/6 536/22.1,23.1 800/205

References Cited [Referenced By]

U.S. Patent Documents

5296462	Mar., 1994	Thomashow	514/2.
5356816	Oct., 1994	Thomashow	435/320.

Other References

Yamaguchi-Shinozaki et al., The Plant Cell, 6:251-264 (1994).
Baker, S.S. et al., Plant Mol. Biol., 24:701-713 (1994).
Jiang, C. et al., Plant Mol. Biol., 30:679-684 (1996).
Horvath, D.P. et al., Plant Physiol., 103:1047-1053 (1993).
Wang, H. et al., Plant Mol. Biol., 28:605-617 (1995).
Okme-Takagi, M. et al., The Plant Cell, 7:173-182 (1995).
Klucher, K.M. et al., The Plant Cell, 8:137-153 (1996).

Wilson, K. et al., The Plant Cell, 8:659-671 (1996).
Li, J.J. et al., Science, 262:1870-1874 (1993).
Guy, C.L., Annu. Rev. Plant Physiol. Plant Mol. Biol. 41:187-223 (1990).
Monroy, A.F. et al., Plant Physiol., 102:1227-1235 (1993).
Monroy, A.F. et al., The Plant Cell, 7:321-331 (1995).
Knight et al., The Plant Cell, 8:489-503 (1996).
Knight, M.R. et al., Nature, 352:524-526 (1991).
Ding, J.P. et al., Plant J., 3:713-720 (1993).
White, T.C. et al., Plant Physiol., 106:917-928 (1994).
Hajela, R.K. et al., Plant Physiol., 93:1246-1252 (1990).
Gilmour, S.J., Plant Physiol., 87:745-750 (1988).
Schiestl, R.H. et al., Current Genetics, 16:339-346 (1989). (Abstract).
Berger, S.L. et al., Cell, 70:251-265 (1992).
Stockinger, E.J., et al., J. Heredity, 87:214-218 (1996). (Abstract).
Walling, L. et al., Nucleic Acids Res., 16:10477-10492 (1988). (Abstract).
Raikhel, N., Plant Physiol., 100:1627-1632 (1992).
Hahn, S., Cell 72:481-483 (1993).
Foster et al., FASEBJ, 8:192-200 (1994).
Ma, J. et al., Cell, 51:113-199 (1987).
Ma, J. et al., Nature, 334:631-633 (1988).
McCarty, D.R., et al., Cell, 66:895-905 (1991).
Guarente, L., Trends Biochem. Sci., 20:517-521 (1995).
Horiuchi, J. et al., Mol. Cell Biol., 15:1203-1209 (1995).
Wolffe, A.P., Trends Biochem. Sci., 19:240-244 (1994). (Abstract).
Brownell, J.E. et al., Cell, 84:843-851 (1996).
Jofuku, K.D. et al., The Plant Cell, 6:1211-1225 (1994).
Elliot, R.C. et al., The Plant Cell, 8;155-168 (1996).
Weigel, D., The Plant Cell, 7:388-389 (1995).
Choi, S.Y. et al., Plant Physiol., 108:849 (1995).

Primary Examiner: Riley; Jezia
Attorney, Agent or Firm: Guerrero; Karen

Goverment Interests

The US government has rights to the present invention under grants from the USDA/NRICGP-.

Parent Case Text

RELATIONSHIP TO COPENDING APPLICATIONS

This application is a continuation-in-part of the following U.S. applications: U.S. application Ser. No.: 09/018,233, filed: Feb. 3, 1998 entitled "ISOLATED DNA ENCODING ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/017,816, filed: Feb. 3, 1998 entitled "CONSTRUCT FOR TRANSFORMING CELL WITH SEQUENCE ENCODING ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/018,235, filed: Feb. 3, 1998 entitled

"ENVIRONMENTAL STRESS TOLERANCE REGULATORY BINDING PROTEIN TRANSFORMED CELL EXPRESSING ENVIRONMENTAL;" now abandoned U.S. application Ser. No.: 09/017,575 filed: Feb. 3, 1998 entitled "STRESS TOLERANCE REGULATORY BINDING PROTEIN;" now abandoned U.S. application Ser. No.: 09/018,227, filed: Feb. 3, 1998 entitled "TRANSFORMED PLANT WITH MODIFIED ENVIRONMENTAL STRESS TOLERANCE GENE EXPRESSION;" now abandoned U.S. application Ser. No.: 09/018,234, filed: Feb. 3, 1998 entitled "METHOD FOR REGULATING EXPRESSION OF STRESS TOLERANCE GENES IN A TRANSFORMED PLANT;" now abandoned and U.S. application Ser. No.: 08/706,270; filed: Sep. 4, 1996, entitled "now U.S. Pat. No. 5,892,009, " each of which are incorporated herein by reference.

Claims

We claim:

1. A plant comprising a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

2. The plant of claim 1, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

3. The plant of claim 1, wherein said polypeptide binds to a member of a class of DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

4. The plant of claim 1, wherein said recombinant molecule comprises a polynucleotide encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

5. The plant of claim 1, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

6. The plant of claim 5, wherein said promoter is regulated by the addition of an exogenous agent.

7. The plant of claim 5, wherein said promoter is a constitutive promoter.

8. The plant of claim 5, wherein said promoter is regulated by changes in environment conditions.

9. A plant comprising a recombinant molecule comprising a polynucleotide that hybridizes to the AP2 domain of a member of the group of sequences consisting of SEQ ID Nos:12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

10. The plant of claim 9 comprising a recombinant polynucleotide comprising a sequence that

hybridizes to a member of the group of sequences consisting of SEQ ID Nos: 12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

11. A method for altering an environmental stress response of a plant, said method comprising (a) providing a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain selected from the group of sequences consisting of SEQ ID Nos:2, 13, 15, 17, 39, 41, 43, 45,47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95; and (b) introducing said recombinant molecule into the plant.

12. The method of claim 11, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

13. The method of claim 12, wherein said polypeptide binds to a member of a class of DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

14. The method of claim 11, wherein said recombinant polynucleotide comprises a sequence encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

15. The method of claim 11, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

16. The method of claim 15, wherein said promoter is regulated by the addition of an exogenous agent.

17. The method of claim 15, wherein said promoter is a constitutive promoter.

18. The method of claim 15, wherein said promoter is regulated by changes in environment conditions.

19. A method for altering an environmental stress response of a plant, comprising (a) providing a recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising a polynucleotide that hybridizes to a member of the group of sequences consisting of SEQ ID Nos: 1, 12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions; and (b) introducing said recombinant molecule into the plant.

20. A recombinant molecule comprising a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% sequence identity to an AP2 domain selected from the group of sequences consisting of SEQ ID Nos: 13, 15, 17, 39, 41, 43, 45, 47,49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

21. The recombinant molecule of claim 20, wherein said polypeptide binds to a cold or dehydration transcription regulating region comprising the sequence CCG.

22. The recombinant molecule of claim 21, wherein said polypeptide binds to a member of a class of

DNA regulatory sequences which includes a subsequence selected from the group consisting of CCGAA, CCGAT, CCGAC, CCGAG, CCGTA, CCGTT, CCGTC, CCGTG, CCGCA, CCGCT, CCGCG, CCGCC, CCGGA, CCGGT, CCGGC, CCGGG, AACCG, ATCCG, ACCCG, AGCCG, TACCG, TTCCG, TCCCG, TGCCG, CACCG, CTCCG, CGCCG, CCCCG, GACCG, GTCCG, GCCCG, GGCCG, ACCGA, ACCGT, ACCGC, ACCGG, TCCGA, TCCGT, TCCGC, TCCGG, CCCGA, CCCGT, CCCGC, CCCGG, GCCGA, GCCGT, GCCGC, and GCCGG.

23. The recombinant molecule of claim 20, wherein said recombinant polynucleotide comprises a sequence encoding a polypeptide that elevates cold-regulated gene levels in the absence of cold acclimation compared with cold-regulated gene levels in a plant lacking said recombinant molecule.

24. The recombinant molecule of claim 20, further comprising a promoter and wherein said polynucleotide is expressed under regulatory control of the promoter.

25. The recombinant molecule of claim 24, wherein said promoter is regulated by the addition of an exogenous agent.

26. The recombinant molecule of claim 24, wherein said promoter is a constitutive promoter.

27. The recombinant molecule of claim 24, wherein said promoter is regulated by changes in environment conditions.

28. A recombinant molecule comprising a polynucleotide that hybridizes to the AP2 domain of a member of the group of sequences consisting of SEQ ID Nos:12, 14, 18, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92 and 94 under high stringency conditions.

29. The method of claim 11, wherein said environmental stress response is a response to cold or freezing.

30. The method of claim 11, wherein said environmental stress response is a response to drought.

31. The method of claim 11, wherein said environmental stress response is a response to salinity.

32. The plant of claim 1, wherein said recombinant molecule comprises a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

33. The method plant of claim 11, wherein said recombinant molecule comprises a polynucleotide that encodes a polypeptide comprising an AP2 domain having at least an 82% ***sequence identity*** to an AP2 domain from the group of sequences consisting of SEQ ID Nos:13, 15, 17, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93 and 95.

United States Patent **6,413,522**
De Villiers-Zur Hausen , et al. **July 2, 2002**

Papilloma viruses, products for the detection thereof as well as for treating diseases caused by them

Abstract

This invention relates to a DNA coding for a peptide of a papilloma virus major capsid protein and a papilloma virus genome, respectively. Furthermore, this invention concerns proteins coded by the papilloma virus genome and antibodies directed thereagainst as well as the use thereof for diagnosis, treatment and vaccination.

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PCT PUB. Date: **June 4, 1998**

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Current U.S. Class: **424/204.1**; 424/199.1; 435/5; 435/69.1; 435/69.3; 435/235.1; 435/320.1; 536/23.72; 530/350; 530/324
Intern'l Class: A61K 039/12; C12N 001/12; C12N 015/00; C07H 021/04
Field of Search: 435/6,701,69.3,69.1,235.1,320.1 536/23.1,23.72 396/2,1 530/350,387.1,324 424/204.1,199.1

References Cited [Referenced By]

Foreign Patent Documents

WO 95/30754	Nov., 1995	WO.
WO 97/04099	Feb., 1997	WO.

Other References

de Villiers et al., 1997, "Prevailing papillomaviurs types in non-melanoma carcinomas of the skin in renal allograft recipients,"International Journal of Cancer 73(3):356-361.
Hagensee et al., 1993, "Self-assembly of human papillomavirus type 1 capsids by expression of the L1 protein alone or by coexpression of the L1 and L2 capsid proteins," Journal of Virology 67(1):315-322.
Kirnbauer et al., 1993, "Effecient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles," Journal of Virology 67(12):6929-6936.
zur Hausen, 1977, "Human papillomavirus and their possible role in squamous cell carcinomas," Curr. Top. Microbiol. Immunol. 78:1-30.
zur Hausen, 1996, "Papillomavirus infections--a major cause of human cancers," Biochimica et Biophysica Acta 1288(2):F55-F78.

Primary Examiner: Salimi; Ali R.
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Parent Case Text

This is a national phase filing of the Application No. PCT/DE97/02659, which was filed with the Patent Corporation Treaty on Nov. 12, 1997, and is entitled to priority of the German Patent Application DE 196 48 962.8, filed Nov. 26, 1996.

Claims

What is claimed is:

1. An isolated polynucleotide consisting essentially of:

(a) a nucleotide sequence encoding the peptide of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8;

(b) a nucleotide sequence hybridizing to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; or

(c) the complement of (a) or (b);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

2. An isolated polynucleotide encoding a peptide of a papilloma virus major capsid protein, wherein the said polynucleotide has been obtained using the following steps:

(a) incubating total DNA isolated from a biopsy of epithelial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

(b) identifying and isolating a polynucleotide that hybridizes to the complement of the nucleotide

sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 in step (a);

wherein the polynucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

3. An isolated polynucleotide, consisting essentially of (a) a nucleic acid encoding a peptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or SEQ ID NO:8, or (b) the complement of (a).

4. An isolated polynucleotide, wherein the polynucleotide consists essentially of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, or the complement thereof.

5. A plasmid comprising the polynucleotide of claim 1 or 2.

6. A plasmid comprising the polynucleotide of claim 3 or 4.

7. An expression vector comprising the polynucleotide of claim 1 or 2.

8. An expression vector comprising the polynucleotide of claim 3 or 4.

9. A host cell comprising the plasmid of claim 5.

10. A host cell comprising the plasmid of claim 6.

11. A host cell comprising the expression vector of claim 9.

12. A host cell comprising the expression vector of claim 8.

13. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 11 under suitable conditions.

14. A method of producing a peptide of a papilloma virus major capsid protein, comprising cultivating the host cell of claim 12 under suitable conditions.

15. A method of detecting a papilloma virus DNA, comprising:

(a) ***hybridizing under stringent conditions*** at least a portion of the polynucleotide of claim 1, 2, 3, or 4 to a DNA sample; and

(b) identifying papilloma virus in said DNA sample by detecting a hybridization signal.

16. A composition comprising the polynucleotide of claim 1, 2, 3, or 4 as reagent for diagnosis and a diagnostically acceptable carrier.

17. A method of producing a papilloma virus genome, comprising:

(a) incubating total DNA isolated form a biopsy of epithlial neoplasm with a nucleic acid having at least a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7, under a condition that allows hybridization of a polynucleotide derived from a papilloma virus genome included in the total DNA to said nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7; and

(b) identifying and isolating a polypnucleotide that hybridizes to the nucleotide sequence of the complement of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7 in step (a).

18. The method of claim 17, wherein the polypnucleotide has a homology of at least 90% to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:4, or SEQ ID NO:7.

19. A composition comprising the polynucleotide of claim 1, 2, 3, or 4 as reagent for vaccination and a pharmaceutically acceptable carrier.

20. A method of vaccinating a subject in need against papilloma virus, comprising administering to said subject the composition of claim 19.

21. A method of diagnosing a condition caused by papilloma virus in a subject in need, comprising exposing said subject the composition of claim 16.

22. A method of using the polynucleotide of claim 1, 2, 3, or 4 as reagent for diagnosis.

23. The method according to claim 22, wherein the diagnosis concerns papilloma virus infections or diseases.

United States Patent **6,372,961**
Tarczynski , et al. **April 16, 2002**

Hemoglobin genes and their use

Abstract

The invention relates to the genetic manipulation of plants, particularly to the expression of hemoglobin genes in transformed plants. Nucleotide sequences for the hemoglobin genes and methods for their use are provided. The sequences find use in enhancing seed germination, seedling growth, and overall growth and metabolism of the plant.

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Filed: **August 17, 1999**

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Intern'l Class:	C12Q 001/68; A01H 005/00; C12N 005/14; C12N 005/82; C07H 021/04
Field of Search:	435/6,320.1,91.1,419,468 536/23.1,24.5,24.3,24.31,24.33 800/295,278,300.1

References Cited [Referenced By]

U.S. Patent Documents

5049493	Sep., 1991	Khosla et al.	435/69.

Foreign Patent Documents

WO98/12913	Apr., 1998	WO	.
WO99/02687	Apr., 1998	WO	.
WO00/00597	Jan., 2000	WO	.

Other References

Sowa et al. Altering hemoglobin levels changes energy status in maize cells under hypoxia. Proc. Natl. Acad. Sci. vol. 95, pp. 10317-10321.*
Landsmann et al., "Common evolutionary origin of legume and non-legume plant haemoglobins", Nature, vol. 324, pp. 166-168, 1986.
Bogusz et al., "Functioning haemoglobin genes in non-nodulating plants", Nature, vol. 331, pp. 178-180, 1988.
Appleby et al., "A role for haemoglobin in all plant roots?", Plant, Cell and Environment, vol. 11, pp. 359-367, 1988.
Bogusz et al., "Nonlegume Hemoglobin Genes Retain Organ-Specific Expression in Heterologous Transgenic Plants", The Plant Cell, vol. 2, pp. 633-641, 1990.

Christensen et al., "Hemoglobin genes in non-legumes: cloning and characterization of a Casuarina glauca hemoglobin gene", Plant Molecular Biology, vol. 16, pp. 339-344, 1991.
Becana et al., "Oxidation and Reduction of Leghemoglobin in Root Nodules of Leguminous Plants.sup.1 ", Plant Physiol., vol. 98, pp. 1217-1221, 1992.
Kennedy et al., "Anaerobic Metabolism in Plant.sup.1 ", Plant Physiol., vol. 100, pp. 1-6, 1992.
Ji et al., "Involvement of Molecular Oxygen in the Enzyme-Catalyzed NADH Oxidation and Ferric Leghemoglobin Reduction.sup.1 ", Plant Physiol.. vol. 100, pp. 33-39, 1992.
Taylor et al., "A cereal haemoglobin gene is expressed in seed and root tissues under anaerobic conditions", Plant Molecular Biology. vol. 24, pp. 853-862, 1994.
Harutyunyan et al., "The Structure of Deoxy-and Oxy-leghaemoglobin from Lupin", J. Mol. Biol., vol. 251, pp. 104-115, 1995.
Jacobsen-Lyon et al., "Symbiotic and Nonsymbiotic Hemoglobin Genes of Casuarina glauca", The Plant Cell, vol. 7, pp. 213-223, 1995.
Hardison, Ross C., "A brief history of hemoglobins: Plant, animal, protist, and bacteria", Proc. Natl. Acad. Sci. USA, vol. 93, pp. 5675-5679, 1996.
Andersson et al., "A new hemoglobin gene from soybean: A role for Hemoglobin in all plants", Proc. Natl. Acad. Sci. USA, vol. 93, pp. 5682-5687, 1996.
Prytulla et al., "Gene synthesis, high-level expression and assignment of backbone .sup.15 N and .sup.13 C resonances of soybean leghemoglobin", FEBS Letters, vol. 399, pp. 283-289, 1996.
Andersson et al., "Cell-Specific Expression of the Promoters of Two Nonlegume Hemoglobin Genes in a Transgenic Legume, Lotus corniculatus", Plant Physiol., vol. 113, pp. 45-57, 1997.
Arredondo-Peter et al., "Molecular Cloning of the Cowpea Leghemoglobin II Gene and Expression of It's cDNA in Escherichia coli.sup.1 ", Plant Physiol., vol. 114, pp. 493-500, 1997.
Nie et al., "Mitochondrial Respiration and Hemoglobin Gene Expression in Barley Aleurone Tissue.sup.1 ", Plant Physiol., vol. 114, pp. 835-840, 1997.
Arredondo-Peter et al., "Rice Hemoglobins.sup.1 ", Plant Physiol., vol. 115, pp. 1259-1266, 1997.
Duff et al., "Expression, Purification, and Properties of Recombinant Barley (Hordeum sp.) Hemoglobin", The Journal of Biological Chemistry, vol. 272, No. 27, pp. 16746-16752, 1997.
Trevaskis et al., "Two hemoglobin genes in Arabidopsis thaliana: The evolutionary origins of leghemoglobins", Proc. Natl. Acad. Sci. USA, vol. 94, pp. 12230-12234, 1997.

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Parent Case Text

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. application Ser. No. 60/097,242 filed Aug. 20, 1998, which is herein incorporated by reference.

Claims

That which is claimed:

1. An isolated nucleotide sequence selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide encoding a hemoglobin polypeptide, said polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through, (C).

2. An expression cassette comprising a nucleotide sequence of claim 1, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a plant cell.

3. The expression cassette of claim 2, wherein said promoter is a tissue specific promoter.

4. The expression cassette of claim 3, wherein said promoter is selected from the group consisting of promoters driving expression in root, seed, embryo, and green tissue.

5. The expression cassette of claim 2, wherein said promoter is a constitutive promoter.

6. The expression cassette of claim 2, wherein said cassette further comprises a chloroplast targeting sequence operably linked to the nucleotide sequence.

7. The expression cassette of claim 6, wherein said promoter is a constitutive promoter.

8. A method for enhancing seed germination and seedling growth, said method comprising transforming a plant with at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through, (C).

9. The method of claim 8, wherein said promoter is a seed-specific or an embryo-specific promoter.

10. The method of claim 9, wherein said promoter is an alpha-amylase promoter.

11. A method for manipulating oxygen concentration in a plant cell, said method comprising transforming said plant cell with at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C).

12. The method of claim 11, wherein said promoter is a constitutive promoter.

13. A transformed plant cell having stably incorporated into its genome at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

b) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

c) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

d) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C);

and wherein said transformed plant cell has been transformed with said nucleotide sequence.

14. The plant cell of claim 13, wherein said promoter is a seed-specific or an embryo-specific promoter.

15. The plant cell of claim 13, wherein said promoter is an alpha-amylase promoter.

16. The plant cell of claim 15, wherein said promoter is a constitutive promoter.

17. A transformed plant having stably incorporated into its genome at least one nucleotide sequence encoding a hemoglobin protein said nucleotide sequence operably linked to a promoter that drives expression in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

a) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

b) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

c) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 or 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

d) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C);

and wherein said transformed plant has been transformed with said nucleotide sequence.

18. The plant of claim 17, wherein said promoter is a seed-specific or an embryo-specific promoter.

19. The plant of claim 17, wherein said promoter is an alpha-amylase promoter.

20. The plant of claim 17, wherein said promoter is a constitutive promoter.

21. The plant of claim 17, wherein said plant is a monocot.

22. The plant of claim 21, wherein said monocot is corn, wheat, rice, barley, sorghum, or rye.

23. The plant of claim 17, wherein said plant is a dicot.

24. The plant of claim 23, wherein said dicot is selected from the group consisting of soybean, non-vegetable brassica, sunflower, alfalfa, cotton or safflower.

25. Seed of the plant of claim 21.

26. Seed of the plant of claim 22.

27. Seed of the plant of claim 23.

28. Seed of the plant of claim 24.

29. A method for modulating hemoglobin levels in a plant cell, said method comprising transforming said plant cell with at least one nucleotide sequence encoding a hemoglobin protein, said nucleotide sequence operably linked to a promoter in a plant cell, wherein said nucleotide sequence is selected from the group consisting of:

A) a nucleotide sequence encoding the amino acid sequence of SEQ ID NOS: 2 or 4;

B) a nucleotide sequence set forth in SEQ ID NOS: 1 or 3;

C) a polynucleotide having at least 70 percent ***sequence identity*** to SEQ ID NOS: 1 and 3, wherein the percent ***sequence identity*** is based on the entire sequence and is determined by GAP analysis using default parameters; and

D) a polynucleotide complementary to the entire length of a polynucleotide of (A) through (C).

30. The method of claim 29, wherein said plant cell is maize.

31. The method of claim 29, wherein said promoter is a heterologous promoter.

32. The method of claim 29, wherein said promoter is a constitutive promoter.

33. An isolated nucleotide sequence having at least 50 nucleotides in length which hybridizes under high stringency conditions, including a wash in 0.1.times.SSC to 60 to 65.degree. C., to a polynucleotide having the sequence set forth in SEQ ID NOS: 1 or 3.

United States Patent **6,274,790**
Kunst , et al. **August 14, 2001**

Nucleic acids encoding a plant enzyme involved in very long chain fatty acid synthesis

Abstract

Nucleic acid molecules encoding an enzyme involved in very long chain fatty acid (VLCFA) elongation in plants are disclosed. The invention includes a cDNA, genomic clone and encoded protein, as well as plants having modified VLCFA composition, such as modified epicuticular waxes, and methods of making such plants.

Inventors: **Kunst; Ljerka** (North Vancouver, CA); **Millar; Anthony A.** (Vancouver, CA)
Assignee: **The University of British Columbia** (Vancouver, CA)
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Field of Search: 800/298,281,264,287 435/69.1,468,419,430,320.1 536/23.6

References Cited [Referenced By]

U.S. Patent Documents

5443974	Aug., 1995	Hitz et al.
5455167	Oct., 1995	Voelker et al.
5530192	Jun., 1996	Murase et al.

Foreign Patent Documents

4433307A	Mar., 1996	DE.
WO95/15387	Jun., 1995	WO.
WO96/13582	May., 1996	WO.

Other References

Benfey et al., Science 250:959-966, Nov. 1990.*
Newman et al., "AC T22193," EMBL Database, Jun. 27, 1994.
Newman et al., "AC T76616," EMBL Database, Mar. 25, 1995.
Sohal & Jenkins, "Epidermal-specific gene expression in Brassica and Arabidopsis," Plant Physiology Supplement, vol. 111, No. 2, p. 6, Jun. 2, 1996.
Evenson & Post-Beittenmiller, "Fatty acid-elongation activity in rapidly expanding leek epidermis," Plant Physiology, vol. 109, pp. 707-716, 1995.
Millar & Kunst, "Very-long-chain fatty acid biosynthesis is controlled through the expression and specificity of the condensing enzyme" The Plant Journal 12(1):121-131, 1997.
Newman et al., "Genes Galore: A Summary of Methods for Accessing Results from Large-

Scale Partial Sequencing of Anonymous Arabidopsis cDNA Clones" Plant Physiol., 106:1241-1255, 1994.
Katavic et al., "In planta transformation of Arabidopsis thaliana " Mol Gen Genet, 245:363-370, 1994.
Lemieux, "Molecular genetics of epicuticular wax biosynthesis" Trends in Plant Science, 1 (9):312-318, 1996.
Kunst et al., "Fatty acid elongation in developing seeds of Arabidopsis thaliana" Plant Physiol. Biochem., 30:(4) 425-434, 1992.
Lassner et al., "A Jojoba .beta.-Ketoacyl-CoA Synthase cDNA Complements the Canola Fatty Acid Elongation Mutation in Transgenic Plants" The Plant Cell, 8:281-292, 1996.
James et al., "Directed Tagging of the Arabidopsis Fatty Acid Elongation 1 (FAE1) Gene with the Maize Transposon Activator," The Plant Cell, 7:309-319, 1995.
James & Dooner, "Isolation of EMS-induced mutants in Arabidopsis altered in seed fatty acid composition" Theor Appl Genet, 80:241-245, 1990.
Lemieux et al., "Mutants of Arabidopsis with alterations in seed lipid fatty acid composition" Theor Appl Genet, 80:234-240, 1990.

Primary Examiner: McElwain; Elizabeth F.
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Parent Case Text

PRIORITY CLAIM

This application claims priority to co-pending U.S. provisional patent application Ser. No. 60/043,831, filed on Apr. 14, 1997.

Claims

We claim:

1. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region hybridezes under stringent conditions to: Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

2. A recombinant nucleic acid molecule according to claim 1 wherein the promoter sequence comprises at least 50 consecutive nucleotides of the sequence shown in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

3. The recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 70% identical to the sequence set forth in Seq. I.D. No. 12.

4. A recombinant nucleic acid molecule according to claim 1, wherein the promoter sequence is at least 80% identical with the sequence set forth in Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

5. A recombinant vector comprising a nucleic acid molecule according to claim 1.

6. A transgenic plant comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.

7. The recombinant nucleic acid molecule according to claim 1, wherein the nucleic acid sequence encodes a protein having very long chain fatty acid elongase activity.

8. A method of producing a transgenic plant comprising introducing into the plant the recombinant nucleic acid molecule of claim 1.

9. A plant produced by sexual or asexual propagation of the transgenic plant produced according to the method of claim 8, or by propagation of progeny of the transgenic plant, wherein the plant comprises the recombinant nucleic acid molecule.

10. A method of isolating a nucleic acid molecule having promoter activity, comprising ***hybridizing under stringent conditions*** a nucleic acid preparation with a probe comprising Seq. I.D. No. 12 or the complement of Seq. I.D. No. 12.

11. A plant cell comprising a heterologous nucleic acid sequence, wherein the heterologous nucleic acid sequence comprises the recombinant nucleic acid molecule of claim 1.

12. A recombinant nucleic acid molecule comprising a promoter sequence operably linked to a nucleic acid sequence, wherein the promoter sequence comprises a transcriptional regulatory region capable of mediating gene expression in epidermal cells of Arabidopsis wherein the transcriptional regulatory region is obtainable from a plant VLCFA condensing enzyme gene comprising an open reading frame that hybridizes under stringent conditions to Seq. I.D. No. 3 or to the complement of Seq. I.D. No. 3.

United States Patent **6,413,752**
Takashima , et al. **July 2, 2002**

Protein capable of catalyzing transamination stereoselectively, gene encoding said protein and use thereof

Abstract

A novel protein from Mycobacterium aurum SC-D423 capable of converting acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine is provided.

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References Cited [Referenced By]

U.S. Patent Documents

5965432	Oct., 1999	Kobayashi et al.	435/280.

Foreign Patent Documents

0 404146	Dec., 1990	EP.
0857790	Aug., 1998	EP.
0987332	Mar., 2000	EP.
61-187786	Aug., 1986	JP.
WO 98 48030	Oct., 1998	WO.
WO 99 46398	Sep., 1999	WO.
WO 00 26351	May., 2000	WO.

Other References

Database WPI Section Ch, Week 198013, Derwent Publications Ltd., London, GB: An 1980-22950C XP002142724 & JP 55022630A (Mitsui Toatsu Chem. Inc.), Abstract.
Database WPI Section Ch, Week 198604, Derwent Publications Ltd., London, GB: An 1986-025607 XP002142725 & JP 60 248647A (Yamakawa Yakuhin KK), Dec. 9, 1985), Abstract.

Shin Jong-Shik et al., "asymmetric synthesis of chiral amines with omega-transaminase." Biotechnology and Bioengineering., vol. 65, No. 2, Oct. 20, 1999, pp. 206-211 XPOO214278 ISSN: 0006-3592 728.

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Claims

What is claimed is:

1. An isolated protein comprising an amino acid sequence represented by SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.

2. An isolated protein comprising an amino acid sequence represented by SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine, and wherein said protein has a single amino acid substitution within Sequence ID No. 1.

3. The isolated protein of claim 2, wherein alanine is substituted for threonine at position number 2 in SEQ ID NO. 1.

4. An isolated protein comprising an amino acid sequence which has at least 60% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine and wherein said protein has a molecular weight of about 37 kDa as a monomer, and is obtainable from Mycobacterium aurum SC-S432.

5. An isolated protein comprising an amino acid sequence which has at least 90% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.

6. The isolated protein of claim 5, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

7. An isolated protein comprising an amino acid sequence which has at least 80% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine, and wherein said protein has a molecular weight of about 37 kDa as a monomer.

8. The isolated protein of claim 7, wherein said protein has at least 90% ***sequence identity*** to SEQ ID NO. 1.

9. The isolated protein of claim 8, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

10. An isolated protein comprising an amino acid sequence which has at least 80% ***sequence identity*** to SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in

the presence of a racemic mixture of sec-butylamine, and wherein said protein is derived from a microorganism belonging to the genus Mycobacterium.

11. The isolated protein of claim 10, wherein said protein has at least 90% ***sequence identity*** to SEQ ID NO. 1.

12. The isolated protein of claim 11, wherein said protein has at least 95% ***sequence identity*** to SEQ ID NO. 1.

13. An isolated protein comprising an amino acid sequence represented by amino acids 23 to 339 of SEQ ID NO. 1, wherein said protein converts acetophenone to an optically active 1-phenylethylamine in the presence of a racemic mixture of sec-butylamine.